Regenerative Medicine in Cardiovascular Diseases
Research and Clinical Application: Coordination in Bratislava Region

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Background

2013 Government adopted the strategic document. 
Research and Development in the Slovak Republic 2020 
in the field of Biomedicine and Biotechnology

Module 5. Regenerative and transplantation medicine

- Novel therapies for patients with injuries, end-stage organ failure, or other clinical problems.

- Scientists in the field of regenerative medicine and tissue engineering are now applying the principles of cell transplantation, material science, and bioengineering to construct biological substitutes that will restore and maintain normal function in diseased and injured tissues. The stem cell field is also advancing rapidly, opening new avenues for this type of therapy.

- While stem cells are still in the research phase, some therapies arising from tissue engineering endeavors have already entered the clinical setting successfully, indicating the promise regenerative medicine holds for the future.
Programs:

2009-2014 Centre of Excellence for Biotechnology and Biomedicine
joint project Comenius University Bratislava and Slovak Academy of Science.

2012-2015 Centre of Competence for Research and Development in Molecular Medicine
joint project Comenius University Bratislava and small to medium-sized enterprise

2013-2015 University Science Park for Biomedicine
Comenius University Bratislava
Slovak Academy of Science

Government supported projects:

- Ministry of Health : National Health Strategy 2013-2020
- Ministry of Education, Science and Sport (APVV and VEGA)

Pharmaceutical industry

- Clinical trials
- Commercial projects
University Science Park for Biomedicine

Slovak Academy of Science

- Biomedical Research Center
- Cancer Research Institute BMC SAS
- Institute for Clinical and Translational Research BMC
- Institute of Experimental Endocrinology BMC
- Institute of Virology BMC
- Institute for Heart Research
- Institute of Experimental Pharmacology & Toxicology
- Institute of Molecular Physiology and Genetics

Comenius University Bratislava

University Science Park primarily focuses on biomedicine, biotechnology, enviro-medicine, as well as the area of societal challenges of the 21st century in bioethics, law and socio-economic rights
The National Institute of Cardiovascular Diseases

The NICD was set up in 1979 and aims to be a world-class centre of excellence in pioneering novel, integrative strategies in preventative and therapeutic cardiovascular medicine. The Institute brings together basic and clinical scientists from academic environment to expert clinicians.

Cardiology and Angiology Clinic Units:
- General Cardiology
- Cardiology and Angiology
- Intensive Angiology
- Acute Cardiology and Coronary Care
- Arrhythmias and Cardiostimulation
- Interventional Cardiology
- Non-invasive Cardiology

Cardiosurgery Clinic
Vascular Surgery Clinic
Heart failure and Transplantation Units
Intensive Care Unit
Interventional radiology Unit
Infrastructure of project

**Experiential labs**
- Pharmacological intervention to process of differentiation of stem cells.
- Pharmacology of signal pathways of ischemic myocardium regeneration and regeneration of critical limb ischemia

**Cardiocentre NICD a.s.**
- Prof. Maďarič: Cell therapy of no options patients with critical limb ischemia
- Dr. Hulman: Cell therapy of chronic ischemic myocardium during the CABG surgery

**Clinical practice**
- Cell therapy as possible therapy for patients.
- Patents for methodology and drugs
- New guideline for regenerative medicine in Cardiovascular system

**International partners**
- Prof. Hening, Pharmacology, Medical Faculty, University of Chronningen: "Microcirculation"
- prof. Wang, Cardiocentrum, Harvard Medical Faculty, Boston: "miRNA and Heart Failure"
- Dr. Bartunek Alstat, Cardiovascular Centre Aalst Belgium: "Stem cell therapy of CVD"

**Certified Centres**
- Certified laboratories of Centres of Excellence and University Science parks CU and SAS for GLP and ISO processes of manipulation of stem cells.

**Purchaser**
- Incubators, spin-off company and startups,
- Contracts with pharma companies and biotechnology SME
- New strategy of patient therapy
Regulatory framework for the development of stem cells therapy

EMA - Committee for Advanced Therapies (CAT)

1. Salmikangas P., Menezes-Ferreira M, ...Kyselovic J,... Schnieder CK: 
   *Manufacturing, characterization and control of cell-based medicinal products: challenging paradigms toward commercial use.* 

2. Schussler-Lenz M, Beuneu C, ... Kyselovic J,...Salmijangas P.: 
   *Cell-based therapies for cardiac repair – overview of scientific observations and regulatory viewpoints* 
   *European Heart Journal*, PMID 26470631, 2015

3. Jekerle V, Palomäki T, ...Kyselovic J,...Salmikangas P: 
   *Critical issues and a regulatory framework for the development of stem cells – based medicinal products – a report from the first European Medicines Agency Workshop.* (in preparation)
Cell Therapy of Critical limb ischemia – Bratislava Cardiovascular Center

- Long term research project: 2009 – 2016 = 115 patients
- Analysis of factors associated with the therapeutic benefit of cell therapy in patients with “no-option“ critical limb ischemia
- Comparison of safety and efficacy of IA verus IMr cells application (Klepanec et al., Cell Transplant 2012)
- Primary end-point: limb salvage + wound healing

This study was created by realization of research project “Transplantation of autologous bone-marrow stem cells in patients with critical limb ischemia”. ITMS code: 26240220023
Characterization of Mesenchymal Stem Cells of “No-Options” Patients with Critical Limb Ischemia Treated by Autologous Bone Marrow Mononuclear Cells

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1 Cancer Research Institute, Slovak Acı Institute, Bratislava, Slovakia, 2 Slovak 1

Abstract

Background: Application of cell therapies in the treatment of critical limb ischemia (CLI) is still in the experimental stage and there are no data about the properties of bone marrow mononuclear cells in CLI patients.

Methods and Findings: In this study, we investigated the properties of bone marrow mononuclear cells in CLI patients. We evaluated the expression of CD34, CD44, and CD133 markers on the cell surface and the proliferative potential of the cells in vitro. We also evaluated the clinical outcome of the transplantation in terms of the relief of symptoms.

Conclusions: The results of this study suggest that bone marrow mononuclear cells can be used as a potential therapeutic option for CLI patients. Further studies are needed to confirm these findings and to evaluate the long-term effects of bone marrow mononuclear cell transplantation.

Intra-arterial Autologous Bone Marrow Cell Transplantation in a Patient with Upper-extremity Critical Limb Ischemia

Juraj Madaric, Andrej Klepanec, Martin Mistrik, Cestmir Altaner, Iv

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No Difference in Intra-Arterial and Intramuscular Delivery of Autologous Bone Marrow Cells in Patients With Advanced Critical Limb Ischemia

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HARVEST SmartPReP2 BMAC System (TERUMO)

Point of Care Separation Technology

Inclusion Criteria:

(1) CLI defined by ankle-brachial index ≤0.4, ankle systolic pressure <50mmHg, toe systolic pressure <30mmHg, transcutaneous oxygen pressure (tcpO₂)<30mmHg

(2) Ischemic skin lesion – CLI Rutherford category 5,6

(3) no option for endovascular or surgical revascularization, or failed revascularization

- Amann B, et al. (Cell Transplant 2009)
- Prochazka V, et al. (Cell Transplant 2010)
- Iafrati MD, et al. (J Vasc Surg 2011)
- Klepanec A, et al. (Cell Transplant 2012)
Advantage of autologous BM-MNCs

Complexity (heterogeneity) of cell product, no immunological issues, extensively tested

- **Hematopoietic cells – EPCs endothelial progenitors** (CD34+, CD133+, VEGFr2)
- **Stromal cells – MSCs** (CD34-, CD45-, CD90+, CD44+, CD105+, etc)
- **Platelets**

Supporting role of CD34- cells in EPCs (CD34+) differentiation and paracrine action

*Cross-talk between cells*

Results

12-month follow-up

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=62)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>7/62 (11%)</td>
</tr>
<tr>
<td>Amputation free survival</td>
<td>39/62 (63%)</td>
</tr>
<tr>
<td>Limb salvage</td>
<td>39/55 (71%)</td>
</tr>
<tr>
<td>Limb salvage + wound healing</td>
<td>33/55 (60%)</td>
</tr>
</tbody>
</table>

66-years old patient – IA BMCs delivery

TCP\(_2\)=10 mmHg

53-years old patient – IM BMCs delivery

TCP\(_2\)=30 mmHg

TCP\(_2\)=8 mmHg
## Results

### Rutherford 5 versus 6 category

<table>
<thead>
<tr>
<th></th>
<th>All patients (n=62)</th>
<th>R5 (n=57)</th>
<th>R6 (n=5)</th>
<th>p (R5 vs R6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>7/62 (11%)</td>
<td>7 (12%)</td>
<td>0</td>
<td>0.92</td>
</tr>
<tr>
<td>Limb salvage</td>
<td>39/55 (71%)</td>
<td>39/50 (78%)</td>
<td>0/5 (0%)</td>
<td>0.001</td>
</tr>
<tr>
<td>AFS</td>
<td>39/62 (63%)</td>
<td>39/57 (68%)</td>
<td>0/5 (0%)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

![Graph showing Amputation-free survival](image)

* p=0.01
Challenges encountered by cell therapy

- Optimal cell typ/source
- Dosing/repeat administration of SCs
- Autologous/Allogeneic MSCs
- Modified SCs
- Mechanism of action

Unresolved questions
Intramyocardial delivery of bone marrow cells and coronary artery bypass grafting (CABG) for chronic ischemic heart disease: safety and efficacy studies.

Clinical Trial: CABG+cell therapy

Type: The CABG+Cell therapy has been designed as a prospective, randomized, open, unicenter trial.

Aims: Providing evidence for the effects of bone marrow stem cell therapy (isolated CD133+ vs heterogeneity cells) and mix of in combination with CABG surgery on ventricular function as well as patients’ clinical outcomes and quality of life.

Patients: 70 for CABG + stem cell therapy a 70 only CABG

Schedule: January 2016 – December 2017
Intramyocardial delivery of bone marrow cells and coronary artery bypass grafting for chronic ischemic heart disease: safety and efficacy studies.

Clinical setting: CD133⁺ Isolation with CliniMACS-unit Miltenyi Biotec

Methods to prepare adult stem cells

MNC's

Magnetic isolation of CD133⁺ bone marrow stem cells
Intramyocardial delivery of bone marrow cells and coronary artery bypass grafting for chronic ischemic heart disease: safety and efficacy studies.
Experimental project:

Synergic regenerative potential of pharmacological intervention to signal pathways in cell based therapy and fibroblast reprogramming in ischemic myocardium

Aims:

1. New strategy for cardiac repair through reprogramming fibroblasts resident in the
2. Regenerative medicine based on cell autologous transplantation modify by pharmacological intervention to potentiate flare-up of bone marrow cells to induce local repair these processes.
3. To monitor regenerative morphological changes in myocardial tissue damage after the pharmacological intervention to certain isolates of bone marrow stem cells.
4. Development of the experimental background for preclinical testing of synergic regenerative potential of the pharmacological intervention to the transcription signal pathways in cell based therapy and fibroblast reprogramming in ischemic myocardium.
Thank you for your attention